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Chapter 30

Intensive culture of scleractinian corals and overview of the techniques used for coral reef exhibits at the Aquarium of the Oceanographic Museum of Monaco. An assessment after 18 years of activity

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ABSTRACT

The first attempts to establish and maintain scleractinian corals in captivity at the Oceanographic Museum of Monaco began in 1989 with the transfer of live specimens from the reefs of Djibouti (eastern Africa) to a single 40 m^3 tank. Today, more than 70 species are kept in 30 tanks of volumes ranging from 0.2 to nearly 130 m^3.

Over the past eighteen years, culturing techniques have evolved continuously. Culture conditions adhere to very strict protocols, particularly with respect to illumination and water quality. Approximately 2000 coral colonies are maintained in culture throughout the year.

In the coral reef tanks presented to the public, the Aquarium implements original techniques both for the rock-work, the sand-bed and the life support systems, with the aim of encouraging the development of the fauna and the flora that are so essential to the balance of the ecosystems.

Our assessment of our experiences with long-term coral culture allows us to draw several interesting conclusions :

- The Aquarium has a proven ability to maintain and propagate original parent colonies over periods of at least eighteen years.
- The culturing procedures, including the process of trimming from parent colonies and producing successive generations of daughter colonies, are effective and sustainable from standpoints of both survival and growth.

Reef ecosystems that develop both in the public area and the culturing facilities over the long term offer multiple advantages :

- They provide a favorable environment to other members of the aquarium reef community and create the most optimal conditions for achieving life cycles as they are understood in natural environments.
- Reconstructing examples of reefs that are similar to their natural counterparts offers visitors a true close-up view of these magnificent ecosystems.
- Particularly important are two unique live features created in 2003 (Around the Lagoon) and 2005 (The Aquarium Nursery) where visitors are invited to understand the basics of the biology of stony corals, the culturing techniques, and the prospects of such global work for a better understanding and conservation of the world's coral reefs.

INTRODUCTION

Reef-building scleractinian corals are among the most difficult marine invertebrates to keep in aquaria. At the Oceanographic Museum of Monaco, they are the keystone species of the live coral ecosystems displayed to the public (Gilles *et al.*, 2004). These exhibits have been made possible by techniques and expertise developed over the past 18 years.

In 2007, more than 2,200 colonies from over 70 species occupy 30 tanks of different volumes,

from a few hundred litres to several hundreds of thousands of litres.
In the Aquarium's Tropical Hall, half of the tanks contain communities of scleractinians, some of spectacular age and size. One *Echinopora* sp. coral has occupied the same place for 18 years and has grown to a diameter of 1 m.
Intensive coral culturing began in 1989 and has made possible these ecosystem-based displays. These results are not simply fortuitous but the fruit of a long and meticulous process, in keeping with the traditions of the Oceanographic Museum.

HISTORIC

In 1914, Dr Öxner, then Director of the Aquarium, introduced the concept of 'living underseascapes'. He put the spotlight on natural environments, rather than individual species, by constructing aquaria that contained both Mediterranean fishes and invertebrates.
From the 1960s until 1988, despite clear progress in keeping invertebrates in general, corals continued to be displayed in aquaria as dead skeletons.

It was not until 1989 that the scleractinian adventure truly began in Monaco. For many years, Professor Jean Jaubert obtained spectacular results in his tank at the University of Nice, thanks to an original process nitrifying-denitrifying water (Jaubert, 1989). Not only did corals survive but they continued growing as shown by a colony of *Turbinaria mesenterina* collected in 1979, and still alive today in the Centre Scientifique de Monaco (Figure 1).
Professor Doumenge, Director of the Museum, then decided to attempt the experiment in large volumes by recreating a 40 m^3 living coral reef based on this principle. The first stage of this project was an expedition to the Gulf of Tadjoura, Djibouti, to collect corals, fishes, invertebrates and rock samples in shallow waters.

Since those pioneering days, the Aquarium has never stopped expanding its expertise. The tanks in which corals are cultured or displayed to the public are operated according to simple principles based directly on observations made on the coral reef.
All the techniques aim for the creation and maintenance of natural equilibria (Figure 2).

THE CASE OF THE GREAT CORAL REEF TANK CREATED IN 2000

This tank occupies the central part of the Aquarium and spans two stories (Ounaïs,

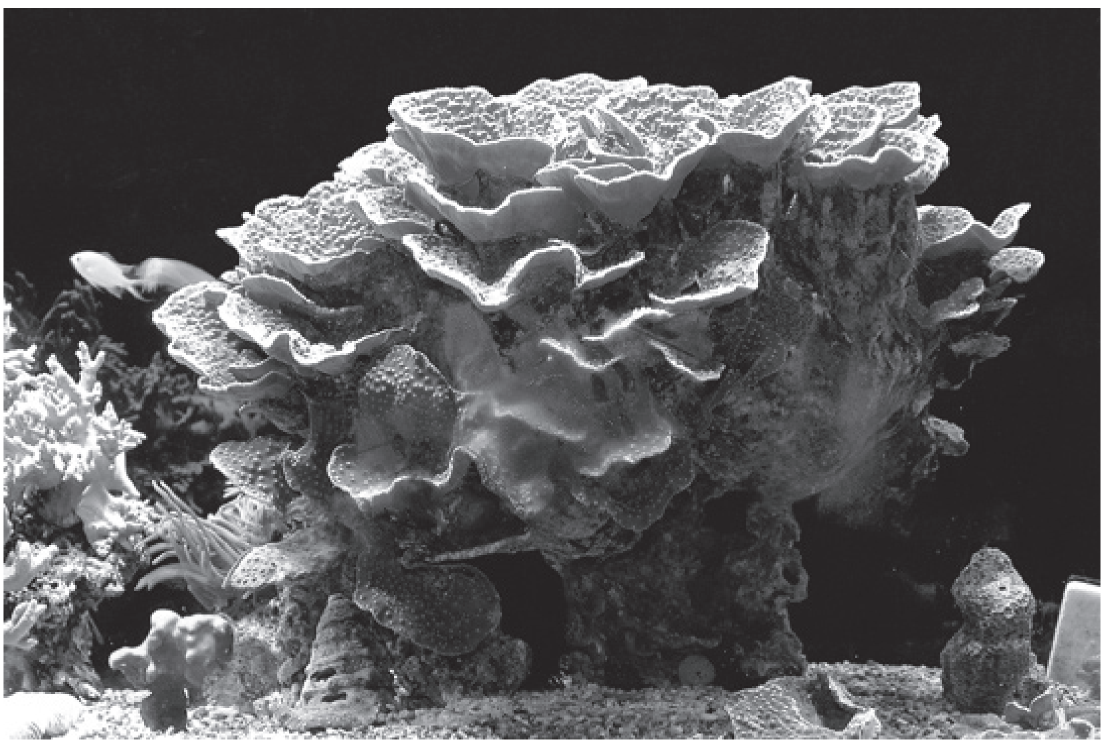

Figure 1: A colony of Turbinaria mesenterina *still alive 30 years after it was collected in the Red Sea*

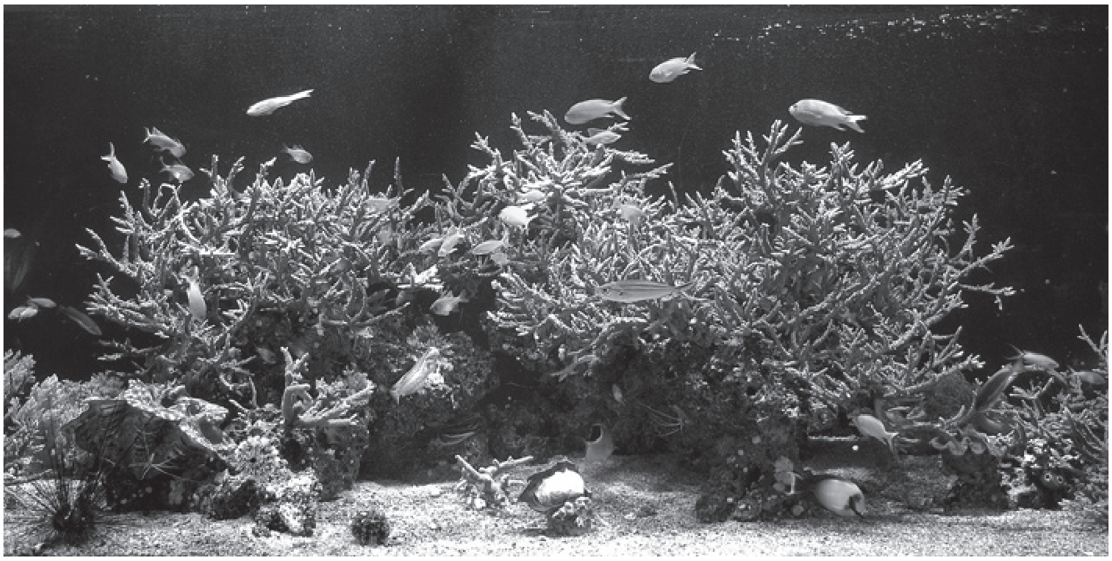

Figure 2 : A perfect equilibrium makes it possible to keep fragile species such as these Acroporids

2000). The aim was to reconstruct a coral reef with a coral lagoon on one side, and the open sea on the other side with the deepest part of the reef and the big predators (Figure 3).

The coral tank is approximately 8 m long and 3 m wide. The water depth is 5.5 m. The total water volume is 115 m^3. The tank can be seen through two main windows, one opening on the upper part of the reef and also showing the technical facilities; the other, opening on the lower part. When standing in front of the big predators window, the visitors can also have a view of the lagoon through two more windows in the wall separating the two areas.

The construction of this tank has been really difficult. The main steps were as follows. After demolishing the existing displays, the floor was stripped down to the bedrock. The former library reading room had to be moved. A trench was sunk through the public area for the passage of fluids from the tank to the filtration room located 30 m away and one story down, and the cliff beneath the structure of the Aquarium had to be reinforced.
The completion of the concrete work took six months. The main difficulty was the access to the job-site, particularly for transporting and installing the acrylic panels. Due to lack of space, no crane could be used.

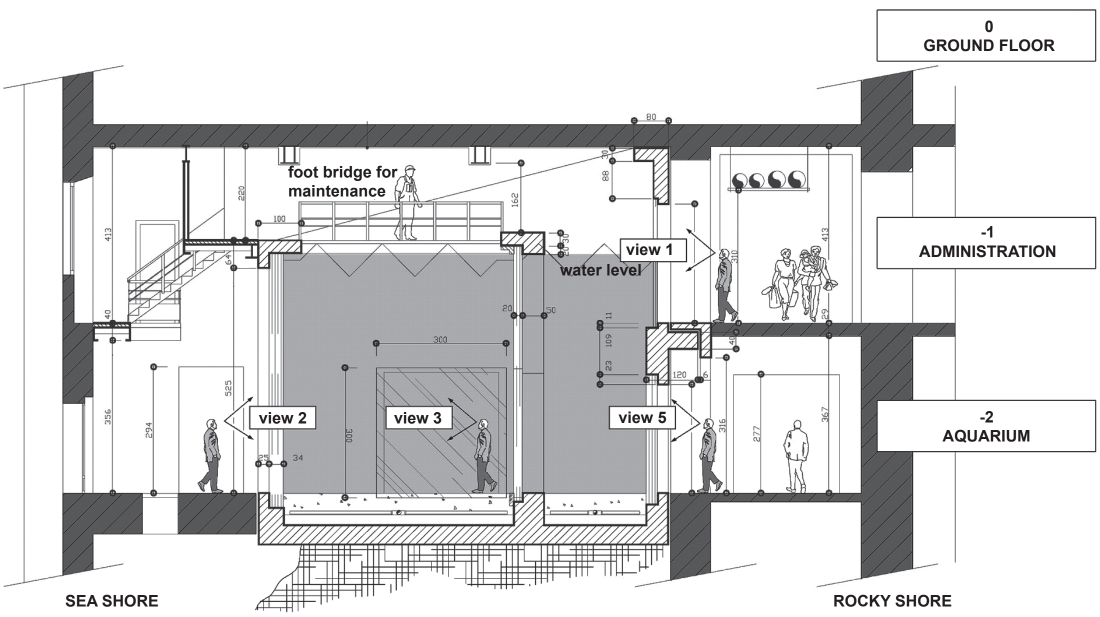

Figure 3 : Lateral view of the Great Coral Reef Tank

The Aquarium is decorated with separate masses of rock made with a structure of plastic mesh supported by resin beams and PVC tubes. A coat of concrete was sprayed on the mesh to form a hard, completely hollow shell. Coral stones of all sizes were placed on this concrete shell. They are cemented together to create something sturdy enough (Figure 4).

Thanks to these stones, the rock-work is natural, is non toxic and constitutes a good substrate for the settlement of corals and sessile fauna and flora.

The water treatment is based on natural systems that have already proven their efficiency in other coral tanks. The coral tank has its own filtration system. A Microcéan system, set up on the bottom of the tank, is made up of two layers of coral sand with different granulometry. These two layers are separated by a sheet of PVC perforated with holes measuring 1 cm in diameter and placed on a grating 14 cm high. The total amount of coral sand weighs 9 metric tons (Figure 5).

The Microcéan system is completed by a biological and mechanical filtration system proportioned for a biomass of 2.5 kg fish.m^{-3}. Every hour, half the water (66 m^3) is filtered through three mechanical filters, each containing 2 metric tons of silica.

Part of the flow (13 $m^3.h^{-1}$) goes through biological filtration made up of two biological filters, each containing 2 metric tons of Biogrog. Before going through the biological filters, oxygen is injected to ensure sufficient oxygen concentration.

The water temperature (25 °C) is regulated through a reversal system able to heat or cool the water on demand. Before returning to the tank, the water is sterilized through an ultra-violet system.

Inside the tank, the water is recirculated at a rate of 100 $m^3.h^{-1}$ thanks to four circulation pumps.

This is a semi-closed system. Raw seawater is injected at a rate of around 1 $m^3.h^{-1}$, that is less than 1 % of the total volume per hour. The water is filtered through two bag filters, varying from 5-25 µm, before going through a UV sterilizer.

As for all the tanks of the Aquarium, follow-up of the physicochemical parameters is rigorous thanks to the centralized management and accurate measurements made using a DR 4000 spectrometer. This regular monitoring validates the operation of the system: for example, total nitrate and phosphate levels remain under 1 mg.L^{-1}.

MANAGENENT OF THE WATER QUALITY OF THE CORAL REEF TANKS

The quality of water is absolutely essential. Most reef tanks receive raw seawater at a variable rate, usually approximately 1 % of the volume per hour. The water intake is located at a distance of 300 m from the shore at a depth of 55 m. At this depth, the water has a salinity of 38.3 ‰ (intermediate between the Red Sea and the Indo-Pacific Ocean), has a temperature rarely exceeding 16 °C and is pollutant free.

Figure 4 (left): Details of the rock-work
Figure 5 (right): The coral sand is placed on the bottom of the tank

Before being introduced into the aquaria, the water is filtered through 25 to 50 µm socket filters or 50 µm sand filters, sterilized with ultra-violet light then filtered at 10 µm through polypropylene cartridges.
To eliminate nitrogen compounds, nitrates in particular, the tanks are equipped with the Jaubert Microcean® system. When properly designed, this system maintains nitrates at a concentration under 1 mg.L^{-1}.

Dynamic mixing, as practised by hobbyists and in other public aquariums, is much reduced in Monaco. In some tanks, water flow is achieved by pumps operating at only two times the volume per hour.
Stable environmental conditions are crucial to the maintenance of hard corals. Since 1995, monitoring of water quality and technical management has been centralized by a network of underwater sensors (Manufacturer: Hach Lange) that make it possible to monitor salinity (model Conductivity probe model 3700 sc PP), dissolved oxygen (model LDO) and pH (model pH-D or model 8350) and temperature (all the model of probes mentionned previously allow the follow-up of temperature) at all times. When threshold values are exceeded, alarms are triggered, alerting staff so they can intervene and correct any problems.
This system also registers the history of each tank and allows correlations to be made between culturing conditions and the status of colonies. To further ensure proper functioning of the entire system, each installation is monitored daily by technicians. They note the temperature and salinity of each tank and check all technical elements. To complete the routine maintenance, nitrate and phosphate concentrations are measured once a month by a spectrophotometer.

In recent years, we have witnessed a major drop in the quality of the water from our pump.
Some events can explain this situation. A semi-floating breakwater 300 m long was built in 2001 and installing it required dredging of large amounts of sediments. Extension works for the port are still underway in 2007 (Figure 6).

Monaco has acquired cutting-edge wastewater treatment facilities. However, in some circumstances like strong rainfall or atypical currents, the water intake absorbs water that has been degraded by physical and chemical pollutants.

Figure 6: Dredging of the sediments near the Aquarium water intake and works still in progress in the Harbour of Monaco in 2007

Many industries have located in the economic activity area of Fontvieille, which probably has a negative affect on the quality of coastal waters. To conclude, a new project for an extension into the sea is planned for the coming decade.

All these factors taken together jeopardize the quality of the water. One consequence is that the most sensitive species are threatened, especially the small polyps species. The phenomenon of chronic pollution is revealed by analysis of sediments caught in settling tanks, with high content, for example, of polycyclic aromatic hydrocarbons, PCB and heavy metals.

Faced with this recurrent problem, the Aquarium has made modifications to remedy this situation. Now, before going into the reef tanks, the raw seawater is filtered through cartridges with activated carbon. As there are very few reliable data on the capacity of carbon to adsorb the contaminants, this system remains empirical. This is why the Aquarium must consider resorting to closed-circuit systems for some reef tanks in the middle term. This would require completely overhauling the technical facilities. A rigorously scientific approach will be necessary to measure the full impact of these changes.

LIGHT MONITORING

The greatest progress over the past 15 years has been made in the handling of light in the aquarium. Corals have specific needs, both quantitatively and qualitatively. Sudden variations or improper exposure may lead to bleaching, metabolic disorders, arrested growth and even death.

Lighting is exclusively artificial and is provided by “daylight”-type metal halogen lamps, ranging from 150 to 2,000 W with a colour temperature ranging from 6,500 to 10,000 K (Some examples : Nepturion HIT-SE 1000 cw 1,000 W 10,000 K by BLV USHIO GROUP ; HQI T 1,000 W Daylight 6,500 K by OSRAM).

The irradiance that reaches the coral is generally between 200 and 800 $\mu E.m^{-2}.s^{-1}$.

The lamps function 13 $h.d^{-1}$. After one year of use, light intensity diminishes considerably, and the spectrum is altered. Thus, to guarantee adequate lighting, the bulbs are changed every year.

To avoid light shock, the lighting is measured before and after bulbs are changed, using a photometer Licor 1400. If necessary, the irradiance is reduced by repositioning the lamps or by using filters.

Figure 7: A colony of Stylophora pistillata *suspended on a nylon thread*

CORAL PROPAGATION AND CULTURING

Coral propagation and culturing at the Oceanographic Museum first began in 1989. Cuttings were periodically taken from certain colonies that were rapidly overgrowing their neighbours, and this regular “pruning” provided a constant source of daughter colonies.

In early trials, cuttings were glued onto neutral supports such as coral stone, PVC tubing or porcelain insulators placed on the bottom of tanks or on PVC “tables” (Ounaïs *et al*., 1993). This technique was far from providing the stability necessary for the colonies.

Over the years, these early techniques have been perfected and incorporated into systematic and reproducible protocols of coral culture. Perfect control of these techniques today enables the production of corals “on demand” to suit the specific biological and aesthetic criteria for each tank.

Several techniques are now in place. For example, fragments are suspended from bars using nylon thread, 0.3 to 0.5 mm in diameter (Figure 7).

The entire water column is utilized, and access to the colonies is easy. The corals can thus be moved and positioned to take advantage of optimal conditions, particularly in terms of lighting.

Another technique involves culturing coral on supports at the bottom of the tank. Each colony must first have a rod of neutral resin sealed directly inside the skeleton with two-component epoxy glue.

One variant involves splitting the rod and simply inserting the coral fragment. The coral regenerates quickly and tissue soon covers the supports (Figure 8). These techniques do not cause much trauma and can be used in all combinations. For instance colonies with rods can be cultured on thread as well.
Cuttings require regular care. Daily inspection ensures their good health and positioning. On a weekly basis, cuttings are removed from the water and cleaned of any algae or organisms.

Growth measurements are made on cuttings to determine their rate of calcification and test their response to new environmental conditions. In this manner, the calcification rate of fragments ranging from 3.6 to 13.5 g for the species *Stylophora pistillata* has been quantified by using the Buoyant Wet Technique (Jokiel *et al*., 1978) at more than 1 % of its dry weight per day (Marchioretti, 1998).

Figure 8: A large colony of Acropora sp. *equiped with a rod of neutral resin sealed directly inside the skeleton*

TRANSFERRING THE COLONIES

Transferring these colonies to display tanks is a delicate operation. For years, corals were fixed to the rockwork or to the walls of the tanks with epoxy resin. Unfortunately, this method anchors them to the substrate so that they cannot be moved later.

Today, the use of colonies anchored to rods has replaced this more permanent method. This technique is an essential element in handling hard corals. In case of poor adaptation to new conditions, or simply if the aesthetic effect is not achieved, the corals can be repositioned or withdrawn at will.

A strict protocol must be observed during transfer. First, the Aquarium team designates on paper the sites on which to place the colonies. Lighting and hydrodynamics are taken into account, as well as the desired aesthetic effect for achieving both realism and natural harmony in the arrangement.

At the selected sites, the substrate is pierced with an underwater pneumatic drill (Manufacturer : Atlas-Copco).

Lighting is measured at each implantation site and on each of the colonies to be transferred. Lighting levels must correspond to culture conditions and under no circumstances can the corals be exposed to excess light.
The colonies are transferred, one by one, to avoid injuring any polyps, thereby optimizing their chances of acclimating.

MANAGEMENT OF HARD CORAL STOCKS

In Monaco, for 9 years now, the day-to-day management of fish is entirely computerized. The management of large invertebrates - such as Echinoderms, Molluscs and Crustaceans - should be fully functional in the coming months. However, the automated management of hard corals remains to be put in place. Several obstacles must be overcome.

The first challenge is the identification of species. This is particularly true for species belonging to the genus *Acropora.* There are many reasons for this :

- Species distinction by external morphology is often impossible.
- Identification by skeletal morphology is something only a few specialists are capable of accomplishing. It is therefore necessary to develop collaborations with specialists in coral systematics.

The second obstacle pertains to improving the specific management of colonies. In effect, individual colonies are very difficult to track over time, for several reasons:

- Whether by pruning or by breakage, colonies regularly separate. Total numbers always vary.
- It is difficult to find a reliable system for marking individual colonies. The labelling systems we have tested until now, such as plastic ties or labels, are not satisfactory. They are either too fragile, they are overgrown rapidly or they become illegible.

Innovative methods must be invented to mark colonies and follow them over time.

USE OF CORAL ECOSYSTEM DISPLAYS IN SCIENTIFIC RESEARCH

The reef ecosystems reconstructed in aquaria are truly natural laboratories in which a range of different studies can be conducted. In Monaco, this research is not conducted by the Aquarium but by affiliated independent laboratories such as the Scientific Center of Monaco. Work is carried out on hard coral ecophysiology, notably on calcification, photosynthesis, respiration and response to ultraviolet radiation.
The Aquarium participates in reciprocal exchanges of information, of biological material, and even technical equipment with this laboratory. One recent example consists of the use of Pulsed Amplitude Modulated fluorometry that aims to detect the quantum yield of photosystem II in photosynthetic organisms, under experimental conditions. This technique is promising, and while its field methodologies in the aquarium remain to be perfected, could allow us in some cases to evaluate the state of coral colonies as well as their state of stress. It would also be possible to detect unhealthy corals and react before it is too late.

These several examples demonstrate the scientific potential of aquarium reef exhibits. This could be taken advantage of more

efficiently by developing interactions and collaborations with the scientific community.

USE OF CORAL REEF DISPLAYS IN TEACHING AND EDUCATION

Coral ecosystems, especially when they are well stocked and vibrant with a rich and colorful fauna, have a powerful impact on the public. The reactions are quite varied, ranging from amazement to reflection to curiosity. The majority of visitors are overcome by an emotion that encourages a willingness to observe and understand.

For over a year now, our animated programs for the public include two live features about corals. The first one, entitled “Around the Lagoon”, consists of a direct dialogue between visitors, presenters and Aquarium technicians (Figure 9).

The public is led to discover corals and coral reefs from a different perspective. One particular emphasis is placed on the threats to these ecosystems and on the need for citizens to adopt a conservation ethic and a proactive behaviour. The same approach is taken with student visitors through an educational workshop entitled “Young Naturalists of the Lagoon”.

On the same principle, visitors have had access to a new activity since the summer of 2006. Guided by a technician and a facilitator, this feature, entitled “The Aquarium Nursery” is broadcast live in the Lecture Hall. The feature shows the different aspects of the breeding of fishes and corals in our facility. An Aquarium technician participates, showing the most recently born young, like seahorses, clownfish, Banggai cardinalfish as well as the “stars” of the programme, which have reproduced for the first time, like the Royal Gramma, *Gramma loreto*.

Coral husbandry has a choice place here. Several approaches are presented: the husbandry techniques, the threats to coral reefs and the role of aquariums and citizens in meeting the challenge of conservation and sustainable development

CONCLUSION

Experiments conducted in recent years in Monaco on Red Sea hard corals demonstrate several important points. Through the rigorous management of culturing conditions and stocking efforts, it is possible to maintain even reputedly sensitive hard corals under long-term culture for as long as 18 years, and up to

Figure 9: The public is led to discover coral reefs through the live feature entitled “Around the Lagoon”

30 years for one *Turbinaria*.

These experiments also show the possible application of these techniques to large volumes : 40 m^3 in 1989 with the Djibouti reef, and 130 m^3 in 2000 with the construction of the Shark Lagoon.

The Aquarium provides all its own coral today and no longer needs to collect specimens from the wild.

The current production system fully satisfies the Aquarium's needs for hard corals, in terms of both quantity and quality. Technically speaking, thanks to reliable and reproducible methodologies, coral culture has become routine and nearly commonplace. Lack of space and personnel are, in fact, the only factors that limit mass production.

Reconstructed biotopes provide reef-dwelling species with ideal living conditions.

Many fish species reproduce regularly in these aquaria, some observed for the very first time in captivity, like the redtooth triggerfish *(Odonus niger)* and the threespot angelfish (*Apolemichthys trimaculatus*). Banggaï Cardinalfish (*Pterapogon kauderni)* juveniles are born and grow without any human intervention (Ounaïs *et al*., 1997).

Regarding our teaching and educational programs, we hope that the emotion we create for our visitors, the experience that they are able to have through their own observations in our Aquarium, the dialogue in which they can participate – these will inspire thoughts or even the willingness to participate in a collective effort to conserve the unique, natural heritage which are the world's coral reefs.

ACKNOWLEDGEMENTS

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APPENDIX I: Fish species and number in the large coral tank

Genus	Species	#	Genus	Species	#
Acanthurus	*blochii*	1	*Naso*	*brevirostris*	1
Acanthurus	*olivaceus*	2	*Naso*	*elegans*	3
Amblygobius	*albimaculatus*	2	*Naso*	*unicornis*	2
Amphiprion	*clarkii*	9	*Naso*	*vlamingii*	6
Amphiprion	*frenatus*	16	*Neopomacentrus*	*cyanomos*	8
Amphiprion	*ocellaris*	62	*Odonus*	*niger*	4
Apogon	*cyanosoma*	18	*Ostracion*	*cubicus*	2
Apogon	*leptacanthus*	3	*Pentapodus*	*emeryii*	1
Apogon	*margaritiphorus*	10	*Pomacanthus*	*imperator*	1
Apolemichthys	*trimaculatus*	2	*Pomacanthus*	*maculosus*	1
Apolemichthys	*xanthurus*	2	*Pomacanthus*	*xanthometopon*	2
Assessor	*flavissimus*	4	*Premnas*	*biaculeatus*	1
Caesio	*lunaris*	7	*Pseudanthias*	*dispar*	44
Caesio	*teres*	46	*Pseudanthias*	*lori*	8
Centropyge	*bispinosus*	2	*Pseudanthias*	*pleurotaenia*	1
Centropyge	*heraldi*	2	*Pseudanthias*	*squamipinnis*	50
Centropyge	*tibicen*	2	*Pseudanthias*	*tuka*	8
Chelmon	*rostratus*	2	*Pseudocheilinus*	*hexataenia*	1
Chromis	*viridis*	50	*Pseudochromis*	*diadema*	4
Chrysiptera	*cyanea*	30	*Pseudochromis*	*porphyreus*	1
Chrysiptera	*hemicyanea*	17	*Pterapogon*	*kauderni*	17
Chrysiptera	*parasema*	21	*Ptereleotris*	*evides*	1
Cirrhilabrus	*cyanopleura*	1	*Ptereleotris*	*microlepis*	4
Cirrhilabrus	*rubripinnis*	4	*Pterocaesio*	*chrysozona*	2
Ctenochaetus	*striatus*	7	*Pygoplites*	*diacanthus*	1
Ctenochaetus	*strigosus*	1	*Salarias*	*fasciatus*	5
Dascyllus	*aruanus*	18	*Scarus*	*quoyi*	3
Ecsenius	*midas*	2	*Scolopsis*	*bilineata*	2
Genicanthus	*lamarck*	2	*Siganus*	*laqueus*	2
Genicanthus	*melanospilos*	2	*Siganus*	*magnificus*	1
Gomphosus	*caeruleus*	3	*Siganus*	*vulpinus*	2
Gomphosus	*varius*	2	*Sphaeramia*	*nematoptera*	16
Hemitaurichthys	*polylepis*	6	*Synchiropus*	*splendidus*	1
Hemitaurichthys	*zoster*	2	*Xanthichthys*	*auromarginatus*	1
Labroides	*dimidiatus*	2	*Zebrasoma*	*scopas*	3
Monodactylus	*sebae*	23	*Zebrasoma*	*veliferum*	3

APPENDIX II: List of the coral species fragmented at the Aquarium of the Oceanographic Museum of Monaco

Family Name	**Species Name**
Acroporidae	*Acropora* sp.
Acroporidae	*Montipora* sp.
Acroporidae	*Montipora digitata*
Agariciidae	*Pavona* sp.
Agariciidae	*Leptoseris* sp.
Cariophyllidae	*Plerogyra sinuosa*
Dendrophylliidae	*Turbinaria peltata*
Dendrophylliidae	*Turbinaria* sp.
Favidae	*Caulastrea furcata*
Favidae	*Favia* sp.
Favidae	*Platygyra lamellina*
Fungidae	*Fungia* sp.
Merulinidae	*Hydnophora rigida*
Merulinidae	*Hydnophora* sp.
Merulinidae	*Merulina ampliata*
Milleporidae	*Millepora dichotoma*
Milleporidae	*Millepora platyphyllia*
Mussidae	*Lobophyllya* sp.
Mussidae	*Symphyllia* sp.
Oculinidae	*Galaxea fascicularis*
Pocilloporidae	*Pocillopora damicormis*
Pocilloporidae	*Pocillopora verrucosa*
Pocilloporidae	*Seriatopora hystrix*
Pocilloporidae	*Stylophora pistillata*
Poritidae	*Porites cylindrica*
Poritidae	*Porites porites*
Siderastreidae	*Psammocora* sp.